

# Microbiological Profile of Human Donor Milk Pre-Pasteurization and Post-Pasteurization in a Comprehensive Lactation Management Centre of a Tertiary Care Hospital in Hanumakonda, Telangana State

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**Abstract:** ***Introduction:** Breast milk is the first nourishment for all humans and mammals. It is the new-born's primary and only source of natural food and nutrients, as well as the development of their immunity. Providing donor human milk to vulnerable neonates who are not having access to their mother's milk, not only saves lives, but also raises breastfeeding awareness and increases breastfeeding rates. This highlights the critical need to develop Human Donor Milk Banks to serve preterm, low birth weight, and ill babies, as well as babies who are unable to breastfeed. The effort of setting up of facility-based lactation management centers is in no way intended to lessen the importance of mother's own milk or the practice of breastfeeding. Microbiological safety of donor human milk (DHM) plays a critical function in assisting vulnerable new-borns and is of greatest priority before issuing the milk to the receiver. **Aim and objectives:** To cater preterm, low birth weight, ill babies and to promote Donor Human Milk Banks which aids in saving the vulnerable neonates and infants. To determine Microbiological safety of Donor mother's milk In a Comprehensive Lactation Management Centre (CLMC) of a tertiary care hospital (GMH) in Hanumakonda, Telangana state. **Materials and Methods:** Prior to donating milk, all mothers were counselled and tested for HIV, HBsAg, HCV and Syphilis / VDRL. For each batch, 1000 ml of milk is pooled, which is collected from different donor mothers and is pasteurized. Both pre-pasteurized and post-pasteurized donor human milk are inoculated on appropriate media, cultured for 18-24 hours, and further processed according to conventional recommendations for organism isolation. **Results:** Prior to and after pasteurization, a total of one hundred milk samples were gathered and submitted. A total of 90 samples were found to contain non-pathogenic organisms in the pre pasteurized samples and no visible growth on culture in the post pasteurized samples. *Serratia* species, *Acinetobacter* species, *Klebsiella* species, growth has been observed in 10 samples. **Conclusion:** Donor human milk from milk banks that is microbiologically healthy can be a lifesaver and immunity booster for life long for preterm, low birth weight neonates who are unable to get their own mother's milk for a variety of reasons. It can also be used to properly nourish and help in growth of these babies. Donor human milk may be safely and successfully prepared to raise public knowledge of the benefits of breastfeeding and the necessity of donating it, to unwell new-borns. This could help lower the under-5 mortality rate in India and around the world.*

**Keywords:** Breast Milk, Donor Human Milk, Preterm Neonates, Microbiological Safety, Lactation Management

## 1. Introduction

Human milk (HM) is the optimal nutrition for all healthy neonates. Tailored to meet each infant's needs, it contains a large variety of nutrients and bioactive components that provide protection from infections and promote an infant's development and growth. According to the American Academy of Pediatrics (AAP), the World Health Organization (WHO), and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), donor human milk (DHM) is considered the best alternative form of nutrition for vulnerable infants, in case of unavailability or insufficiency of own mother's milk<sup>3</sup>. Breastfeeding is the most natural, inexpensive, environment friendly, and easily accessible method to provide all children, rich or poor, with the healthiest start to life and ensures that all children survive and thrive.

Breast milk offers the ideal source of nutrition for the first 6 months after birth, and may remain a part of an infant's diet for the first 2 years of age and beyond<sup>7</sup>. However, many infants lack access to their mother's own milk because of issues related to the mother's illness or death, abandonment, infant's illness, inability to latch, or delay in milk production. This lack of access to breast milk leaves infants more vulnerable to disease, poor health, or death, especially when they are born preterm, have low birthweight, or are severely malnourished<sup>2</sup>.

In 2020, 19.8 million newborns, an estimated 14.7 percent of all babies born globally that year, suffered from low birth weight (< 2500 grams). Globally, an estimated 13.4 million babies were born preterm in 2020 (before 37 completed weeks of gestation). And the total number of under-5 deaths globally was 5 million in 2020. With the birth of 25 million children

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each year India accounts for nearly one fifth of the world's annual child births. 3.5 million babies are born prematurely and 18% are of low birthweight<sup>1</sup>. As part of integrated newborn care, human milk banks reduce death and illness as well as lower health-care costs. Data exist, which support the health benefits of donor human milk, especially for infants born prematurely, with birthweight less than 1,500g, and for infants born in resource-limited settings where a non-breastfed child's risk of death is 6 times that of a breastfed child<sup>4</sup>. Providing donor human milk to vulnerable neonates without access to mother not only saves lives but also enhances awareness about breastfeeding and improves breastfeeding rates. This increase in breastfeeding rates is important because it has the potential to prevent 820,000 "under-5" deaths (i.e., death before age 5 years), of which 87% are infants younger than 6 months of age. Improving breastfeeding rates worldwide is a fundamental driver to achieve Sustainable Development Goals by 2030<sup>6</sup>.

This precious breast milk is not available or cannot be utilized by few neonates due to some of their own medical conditions or due to unavailability of mother's milk due to some reasons. A lactating woman who is willing to donate her surplus expressed breast milk (EMB) should be considered as a potential donor. This brings immense need to establish Human Donor Milk Banks to serve the preterm, low birth weight and sick babies and the babies who couldn't be breast fed. In 2020, it was estimated that there were 756 milk banks in 66 countries, with an increasing number of milk banks being established in low- and middle-income countries. India now has nearly 80 milk banks, operational as per the National Guidelines on Lactation Management Centers in Public Health Facilities<sup>5,8</sup>. In Telangana state, India, there are 4 CLMC centers.

## 2. Materials and Methods

### Materials:

- 1) Donor Human Milk Pre pasteurized - 5 ml
- 2) Donor Human Milk Post pasteurized -5 ml
- 3) Appropriate media such as Blood agar, CLED agar and MacConkey agar according to CLMC guidelines

### Methods:

CLMC and Lactation management unit for quality assurances and safety of DHM for consumption of babies, will be monitored by Hazard analysis critical control point (HACCP) system. Donor mothers were counselled, Consent was taken and screened for the viral markers. Breast milk is then expressed into sterile 30ml bottles, by using breast pump under strict aseptic precautions. Thus, expressed milk from different donor mothers are divided into batches. Each batch consists of 4 donors, whose expressed milk is pooled in a sterile conical flask for further processing. Air surveillance of the collection room, processing room and storage room are done by using Air sampling machine to ensure possibly contamination-free zone.

Pre-pasteurized milk sample is collected into a sterile universal container and the batch undergoes Holder method of pasteurization in which the aliquots undergo heat cycle at

62.5 degree Celsius for 30 minutes and rapid cooling to 4 degrees Celsius, after proper labelling the bottles with batch and pasteurization cycle details. Post pasteurization sample is obtained from the batch and collected in a sterile universal container. And the pasteurized bottles are stored at -20 degree Celsius.

The pre-pasteurized and post pasteurized donor human milk samples are received in microbiology laboratory.

**Pre-pasteurized sample and post-pasteurized sample:** 0.6 ML milk is inoculated by using sterile inoculating 4mm loops, on to the Blood Agar, MacConkey agar, Nutrient agar and Cysteine lactose electrolyte-deficient (CLED) medium with Andrade indicator.

**Incubation:** The plates are incubated for 24 hours at 37 degrees Celsius. After 24 hours the plates are observed for any bacterial growth.

**Observation:** After 24 hours of incubation, the plates are examined for bacterial growth. Pre-pasteurized DHM Cultures showed the growth of the bacterial colonies. Smears from all the culture plates, where growth is present, are done and are subjected to Gram staining.

The gram-stained smears are observed under 100x Oil Immersion. Preliminary tests like Catalase test, Oxidase test and Hanging drop are done and the results are interpreted. Biochemical tests like Indole, Citrate, Triple sugar iron test, Bile esculin, Mannitol, Tube coagulase were performed depending on the preliminary tests. Out of 100 samples, four pre pasteurized DHM cultures and one post- pasteurized have showed growth of pathogenic organisms the rest 99 were sterile.

### Interpretation:

**For pre-pasteurized DHM:** Milk is discarded if samples exceed a count of  $10^4$  CFU/ml for pathogenic organisms and *Staphylococcus aureus*.

**For Post-pasteurized milk:** Milk that has total viable microbial count of 10 CFU/ml or more should be discarded.

## 3. Result

Mothers who accepted to donate milk were counselled to undergo screening test for HIV, HBsAg, HCV and Syphilis before expression of milk Table 1.

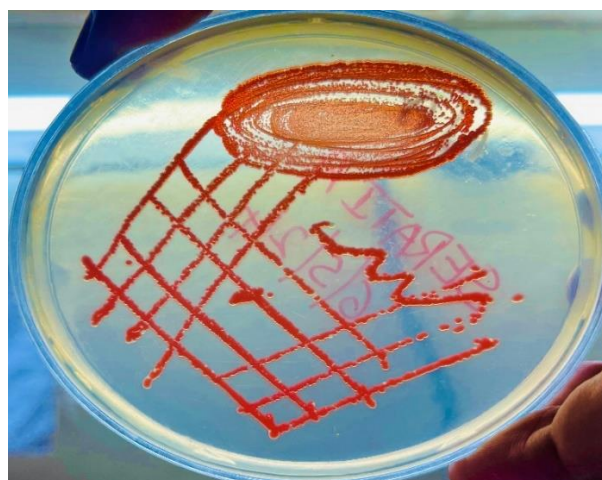
All the 100 milk samples were undergone pasteurization and both Pre & Post pasteurized samples were sent, out of which 90 milk samples have shown isolation of non-pathogenic organism/surface commensal in pre-pasteurized milk and No growth in post pasteurized milk. 6 milk samples have shown growth of *E.coli* and *klebsiella* species in pre pasteurized samples. 1 sample showed growth of *Acinetobacter* species, 1 sample *serratia* species and 2 samples *klebsiella* species in both pre and pasteurized samples. Table 2, Figure 1.

**Table 1:** Serological test which were performed to screen DHM

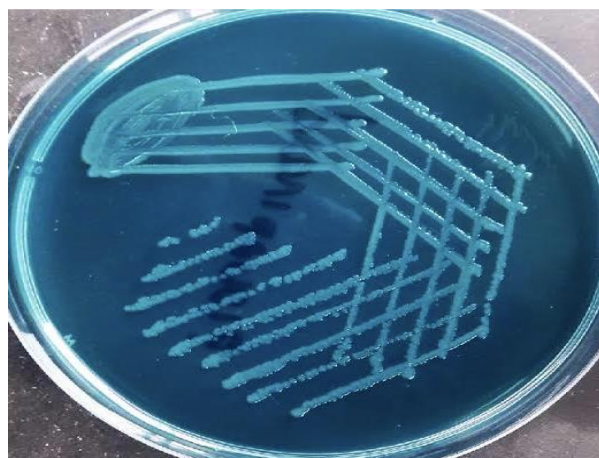
Total No. of Patients	Serological Test			
	HIV	HBsAg	HCV	Syphilis (VDRL)
N=100	Non-Reactive	Negative	Negative	Negative

**Table 2:** Statistics of Processed Milk

Total No. of Samples, N=100	Pre-Pasteurized Milk	Post-Pasteurized Milk
In 90 samples	No Pathogenic organism isolated	No Growth
In 6 samples	E.coli and Klebsiella species	No Growth
In 1 sample	Acinetobacter Species	Acinetobacter Species
In 1 sample	Serratia Species	Serratia Species
In 2 samples	Klebsiella species	Klebsiella species



**Figure 1:** Serratia species



**Figure 2:** Acinetobacter species

#### 4. Discussion

Comprehensive lactation management centers play a pivotal role in saving preterm, premature sick babies who does not have access to their own mother’s milk or due to other medical conditions. Microbiological safety of the Donor mother’s milk is of utmost priority before issuing the milk for consumption. The milk which was collected from CLMC centers were subjected to Holder’s method of pasteurization. Both pre pasteurized and post pasteurized milk samples were subjected to conventional culture methods. In present study 100 cycles of milk pasteurization was done out of which 4 cycles showed growth in post pasteurization milk culture. The isolated organisms are Klebsiella pneumoniae, Serratia species and Acinetobacter species. 10 cycles showed growth in pre pasteurized milk and 4 cycles showed growth of

pathogens in both pre and post pasteurized culture and thus 4 cycles of milk were discarded.

Following are the comparisons of the present study with previous studies

S. No	Author & Year of study	Organism isolated in Prepasteurized sample	Organism isolated in Post pasteurized sample
1	Current study N= 100	Klebsiella pneumoniae-1-1% Serratia species Acinetobacter species.	Klebsiella pneumoniae, Serratia species Acinetobacter species.
2	Pramoda et al. <sup>10</sup> 2020. N=20	Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa.	-
3	Kaur R et al <sup>11</sup> 2020 N=466	MSSA-51-18.3% MRSA -30-10.7% CONS-87-31.3% Micrococcus-80-28.7% E.coli-2-0.7% Klebsiella spp-8-2.8% Acinetobacter spp-2-0.7% ASB-18-6.5%	-
4	Neha gupta et al <sup>9</sup> 2017 N=130	Staphylococcus aureus -15(25%) S.epidermidis-26(43%) E.coli-6(43%) E.aeruginosa-3(21%) K.oxytoca-5(36%) Pseudomonas-4(6%) Molds and yeasts-2(3%)	S. aureus in 3 (25%) E. coli in 2 (17%) Pseudomonas in 2 (16%) S. epidermidis in 5 (42%)

## 5. Conclusion

Donor human milk from milk banks that is microbiologically healthy can be a lifesaver for preterm, low birth weight neonates who are unable to get their own mother's milk for a variety of reasons. It can also be used to properly nourish and help in growth of these babies. Donor human milk may be safely and successfully prepared to raise public knowledge of the benefits of breastfeeding and the necessity of donating it, if practical, to unwell new-borns. This could help lower the under-5 mortality rate in India and around the world.

### Abbreviations:

CLMC- Comprehensive Lactation Management Centre,  
DHM – Donor Human Milk, EBM – Expressed Breast Milk

### Ethical Approval and consent to participate:

The ethical approval for this study has been taken from Kakatiya Institutional Ethics Committee No: ECR/840/Inst/TG/2016/RR/20/62, Kakatiya medical college before sample collection and processing. Consent was obtained from participants.

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**Conflict of Interest:** NIL

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